Seasonal influenza vaccine effectiveness in people with asthma: a national test-negative design case-control study

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Summary: This test-negative design case-control study found that over six seasons, influenza vaccination protected people with asthma against laboratory-confirmed influenza. Protection was highest amongst younger adults (aged 18-54 years). For older adults (>65 years) protection was only provided against influenza B.

Abstract

Background

Influenza infection is an important trigger of asthma attacks. Influenza vaccination has the potential to reduce the incidence of influenza in people with asthma, but uptake remains persistently low, partially reflecting concerns about vaccine effectiveness (VE).

Methods

We conducted a test-negative design case-control study to estimate the effectiveness of influenza vaccine in children and adults with asthma in Scotland over six influenza seasons (2010/11 to 2015/16). We used individual patient level data from 223 primary care practices which yielded 1,830,772 patient-years of data, which were linked with hospital and virological (n=5,910 swabs) data.

Results

Vaccination was associated with an overall 55.0% (95% confidence interval (CI): 45.8-62.7) reduction in the risk of a laboratory-confirmed influenza infection in people with asthma over the six seasons. There was substantial variation in VE between seasons, influenza strains and age groups. The highest VE (76.1%; 95% CI: 55.6-87.1) was found in 2010/11 season where the A(H1N1) strain dominated and there was a good antigenic vaccine match. High protection was observed against A(H1N1) (e.g. 2010/11: 70.7%; 95%CI: 32.5-87.3) and B strains (e.g. 2010/11: 83.2%; 95%CI: 44.3-94.9), but there was lower protection for the A(H3N2) strain (e.g. 2014/15: 26.4%; 95%CI: -12.0-51.6). The highest VE against all viral strains was observed in adults aged 18-54 years (57.0%; 95%CI: 42.3-68.0).

Conclusion

Influenza vaccination gave meaningful protection against laboratory-confirmed influenza in people asthma across all six seasons. Strategies to boost influenza vaccine uptake has the potential to substantially reduce influenza triggered asthma attacks.

Keywords: influenza, vaccination, asthma, laboratory confirmed influenza,

INTRODUCTION

Seasonal influenza results in substantial global morbidity and mortality each year [1, 2]. In people with asthma, influenza infection can exacerbate asthma symptoms, which may result in asthma attacks necessitating medical attention and in many cases hospital admission [3]. The World Health Organization (WHO) and national immunisation programmes recommend annual influenza vaccination in people with asthma as the main prophylactic measure against influenza [4-6]. In the UK, however, vaccination rates in asthma remain well below the 75% uptake target set by the WHO [7]. This suboptimal vaccine uptake is at least in part due to uncertainty amongst people with asthma and healthcare providers around the effectiveness of influenza vaccines [8]. In the UK, suboptimal vaccine protection is partly addressed with the introduction of new vaccine formulations. Specifically, in season 2018/19 younger adults aged 18-64 belonging to an at-risk group were offered a quadrivalent inactivated vaccine (QIV) and people over 65 years old were offered adjuvanted trivalent inactivated vaccine (TIV) [9]. The QIV aims to provide better protection by including two influenza B subtypes given that influenza B affects younger age groups. The adjuvanted TIV aims to enhance the immune response in older people and improve the current suboptimal vaccine effectiveness (VE) observed from traditional TIVs [9].

Our recent systematic review suggested that the vaccine is effective against influenza infection in asthma [8]. However, the conclusions of this review were based mainly on studies in which the overall quality was rated as low due to methodological issues related to the study design and conduct [8]. Two case-control studies published after the search date of the literature review assessed VE in individuals with asthma [10]. The first study compared

the VE between asthma and non-asthma hospitalised patients for laboratory-confirmed influenza [10]. However, there were too few patients with asthma and thus, the study was under-powered to determine the effectiveness of the influenza vaccine [10]. In the second study, the VE was assessed in children 6-59 months during four seasons by various characteristics including asthma [11]. VE of 43.3% was found in the asthma subgroup. However, no further analyses in relation to other demographics or other characteristics related to influenza infection or the vaccination were performed for the asthma subgroup [11].

Most national immunisation committees assess the VE based on evidence from observational studies rather than placebo randomised controlled trials, which are no longer conducted in asthma since the vaccination is now a public health recommendation for all at-risk groups such as people with asthma [12]. Thus, we employed a test-negative design (TND) case-control study to best determine the VE for each influenza season since it is now seen as the gold standard for generating unbiased VE estimates [13-16]. In addition, the large sample size of our TND study using swab samples for multiple seasons enabled us to assess various factors that affect VE in observational studies such as asthma population characteristics and influenza circulating types and subtypes which were not assessed in previous studies due to sample size limitations [8].

The aim of this study was to assess VE in children and adults with asthma. More specifically, the objectives of this study were to: (1) evaluate seasonal influenza VE across and in single seasons; (2) evaluate the VE against common seasonal circulating viral strains; and (3) provide VE estimates by age groups.

METHODS

Study Design

We undertook a retrospective observational TND case-control study to evaluate influenza VE on patients that seek care for acute respiratory infection. In a TND study, cases are those testing positive for influenza and controls are those testing negative for influenza. This study included children (>6 months old) and adults who were recommended by UK immunisation guidelines to receive influenza vaccination i.e. those treated for asthma requiring continuous or repeated use of inhaled or systemic corticosteroids and/or with previous exacerbations requiring hospital admission. The study participants were identified from 223 general practices (sentinel and non-sentinel) and hospitals for acute respiratory illness from influenza season 2010/11 to 2015/16 in Scotland. Patients were swabbed and tested for influenza using the multiplex real-time polymerase chain reaction (RT-PCR) assay [17]. Patients with a positive test for influenza were classified as cases, while those with a negative test were classified as controls. In patients with more than one positive test for influenza, only the first positive test was counted as a single case. VE was estimated by comparing positivity proportions between the vaccinated and unvaccinated patients [16].

The VE assessment in the asthma population was an objective of the Seasonal Influenza Vaccine Effectiveness (SIVE) II project [18] (see included datasets in Figure 1). See Supplementary Appendix 1 for details.

Exposure and Outcome Assessment

The exposure status was based on vaccination administrated between the pre-influenza season and the end of the influenza season. Individuals vaccinated from 1 September until the end of the influenza season defined the "exposed" group. Individuals with no vaccination record, being vaccinated after being tested for influenza, or vaccinated within 14 days prior to testing were also classified as the "unexposed" group [18].

General practices who were part of the sentinel scheme obtained nasal or nasopharyngeal swabs from patients with influenza-like illness (ILI) symptoms. General Practitioners usually collected swab samples from patients presenting with ILI symptoms (independent of whether the patient has or has not been vaccinated) within seven days of date of onset of those symptoms. Each general practice collected up to five samples per week and submitted these to the West of Scotland Specialist Virology Centre (WoSSVC) [18]. Each swab sample collected in general practice sentinel settings was tested by the WoSSVC using the multiplex RT-PCR test for a range of respiratory pathogens, including influenza [18]. For non-sentinel practices or secondary care, other laboratories were involved in testing. Subtype and genetic characterisation was performed for positive influenza sentinel samples and most of non-sentinel general practice and hospital samples. Data on laboratory tests carried out in non-sentinel primary and secondary health care facilities were also collected by the ECOSS database. See Supplementary Appendix 2 for baseline characteristics description.

Statistical Analysis

Baseline characteristics of study participants were described. The relation between vaccination status and baseline characteristics was also provided for cases and controls. Proportions and odds ratios (ORs) were used to describe differences between study groups depending on the nature of each variable. All baseline population characteristics were presented as categorical variables and the x^2 test was used to describe any association in relation to exposure or outcome. Any missing data were reported. All tests were two-tailed and results considered significant if $P<0.05$. See Supplementary Appendix 3 and 4 for details on unit of analysis and meta-analysis.

Primary and Secondary Analysis

Pre-specified subgroup analyses as per our published protocol included the provision of VE for influenza A and B strains per season [18]. Post-hoc analysis not specified in our protocol were also carried out in this study. Specifically, we stratified the VE by age groups in order to investigate the age where immunosenescence begins in adults.

Vaccine Effectiveness

VE and their 95% CIs were estimated using the formula VE = (1- aOR) x 100 based on adjusted OR (aOR) [18]. ORs were calculated by the regression coefficients of vaccine status in the model. A generalised additive logistic regression model was used to explain the relationship between influenza infection and influenza vaccine in presence of other confounding covariates. The model provided VE estimates adjusted for the effects of the covariates: time, age, underlying medical conditions and the source of swab sample collection which were either statistically or epidemiologically associated with the outcome. Adjustment for time was performed for all VE estimates. Time was measured in days from the beginning of October each season. It was included as a spline function to account for bias related to time differences between influenza circulation and vaccine administration during each season [18].

Sample Size

Using data from a previous study [18] we anticipated 1454 asthma patients would be swabbed over the two seasons 2014-2016. We assumed that 582 or 40.0% (1,454*0.40) of asthma patients had been vaccinated for influenza and the number of tests positive for influenza was 218 or 15.0% (1,454*0.15), which gave an 80.0% power to detect a VE of 33.0% [18]. The study recruited 1413 patients swabbed in 2014/15 and 1670 in 2015/16. Sample size details are provided in Supplementary Appendix 5 and statistical analyses were carried out using R Version 3.4.2 and RStudio (Version 1.0.143) [19] within the NHS Scotland data safe haven.

Ethical Considerations

The Privacy Advisory Committee of the Information Services Division, NSS, approved the linkage and the statistical analysis of the anonymised data used in this study.

Reporting

We used the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and REporting of studies Conducted using Observational Routinely-collected Data (RECORD) checklists to guide transparent reporting of this TND case-control study (See Supplementary Appendix 6 and 7) [20-21].

RESULTS

A total registered primary care asthma population of 1,830,772 person-years out of 194,319 people with asthma was recruited in this study over a 16-year period. These data were collected as part of the SIVE II study [18] and included a total of 5,910 swab samples taken from 5,022 asthma patients from 2010/11 to 2015/16 (Figure 2). These swabs were carried out in primary or secondary care settings from people with asthma and tested for influenza with the RT-PCR test. There were 781 of 5,910 (13.2%) swabs tested positive for influenza and were classified as cases (Table 1) and (86.8%) tested negative for influenza and were classified as controls (Figure 2). Patients more likely to test positive for influenza were aged 45-64 years (15.1%), lived in remote small towns (>10%), with no previous seasonal influenza vaccine (15.2%) and had a swab sample collected from a primary care setting (16.7%) (Table 2).

Vaccine effectiveness by season and influenza type and subtype

The VE for the commonly influenza circulating strains was estimated for each strain in each influenza season.

In 2010/11, the overall VE was high (76.1% [95%CI: 55.6-87.1]) with A/H1N1 and B predominating (Table 3). In 2011/12, the overall VE was lower and imprecise (45.1%

[95%CI: -35.1.1-77.7]) with A/H3N2 predominating. Slightly higher and more precise overall VE of 45.2% (95%CI: 13.8-65.1) was observed in the 2012/13 season where all influenza A subtypes co-dominated. The overall VE in 2013/14 (where the predominant strain was A/H1N1) was 52.3% (95%CI: 6.5-75.6). In 2014/15, an overall VE of 48.6% (95% CI: 27.8-63.4) was found with a high swab positivity (16.4%) and predominant strains H3N2 and B. In 2015/16 season, the overall VE of 57.8% (95%CI: 40.1-70.3) was higher compared to previous seasons (apart from 2010/11), the predominant strains were A/H1N1 and B and the swab positivity was 12.0%.

In 2010/11, we found high VE for the influenza A(H1N1) subtype and B with estimates of 70.7% (95% CI: 32.5-87.3) and 83.2% (95% CI: 44.3-94.9) respectively (Table 3). In 2011/12 season a small number of cases of influenza A(H3) subtype and B resulted in low and imprecise VE estimates of 3.7% (95%CI: -240.5-75.0) and 71.8% (95%CI: -358.1-98.3) respectively. In the 2012/13 season where all influenza A subtypes co-dominated, a particularly high VE of 77.5% (95% CI: 9.8-94.4) was observed for A(H1N1), but lower VE was found for the co-circulating influenza A(H3) and B strains. In 2013/14 a VE of 32.0% (95%CI: -52.2-69.6) was observed for the influenza A(H1N1) subtype - imprecision in the VE estimate was due to low swab positivity. In 2014/15, a high VE of 77.0% (95% CI: 53.9-88.5) for influenza B was found. In 2015/16 the swab positivity was 12.0% and a VE estimate of 54.7% (95% CI: 19.5-74.5) was observed for influenza B.

Pooled vaccine effectiveness by influenza type and subtype

The overall VE estimate was 54.9% (95% CI: 44.4-63.4) against influenza A and B types as it is shown by the OR provided in the random effect model (Figure 3). Heterogeneity for this pooled estimate was detected but it was small. A substantially low VE estimate of 29.3% (95% CI: 1.0-49.4) was detected for influenza A(H3), but no heterogeneity was found (Figure 4). A higher pooled VE of 48.4% (95% CI: 19.4-66.9) was found against influenza A(H1N1)

compared to influenza A(H3). Low-to-moderate heterogeneity was observed across seasons (Figure 5). The highest pooled VE of 60.8% (95% CI: 31.6-77.5) was detected for influenza B. Higher heterogeneity was also observed for the influenza B compared to other strains but it was non-significant. Unadjusted OR was used for the 2013/14 season due to zero adjusted OR. This happened due to low circulating levels of influenza B strains resulting in small to zero OR which would have prohibited the provision of any meaningful OR in the meta-analysis and a subsequent VE estimation (Figure 6).

Vaccine effectiveness by age group

This analysis showed that the VE was low in those 55 years and above against influenza A subtypes (except 65-74 years old for A(H1N1)) while high VEs for influenza B were found. VE was high in children (<18 years old) with a VE of 90.5% against A(H1N1) (Table 4).

DISCUSSION

During six influenza seasons influenza vaccination effectiveness was over 50% for laboratory-confirmed influenza in people with asthma. Better protection was observed during seasons with good antigenic match and against the A(H1N1) and B strains. Moderate VE was found against influenza A(H1N1) (47%) and influenza B (62%) and VE was low for influenza A(H3N2) (34%). The seasonal influenza vaccine provided protection in younger adults (aged 18-54 years old) against influenza A(H1N1), influenza A(H3) subtypes and influenza B.

The highest VE was observed in 2010/11 which was characterised by high influenza activity predominated by the influenza A(H1N1) and B strains in the UK [22-23]. While low VE was detected in 2011/12, this is likely due to low and late activity of the predominant A(H3) strain and an antigenic vaccine mismatch [24]. Intra-seasonal VE waning and low VE against the

A(H3N2) was observed in the UK and US [8, 25-26]. This lower VE has been attributed to antigenic drift [26]. In the 2012/13 season, good protection was only found against the A(H1N1) strain; this was consistent with the findings from another UK study [27]. Antigenic drift for influenza B and VE decline for influenza A(H3N2) particularly in the second trimester following vaccination was noted during this season [27-28]. A US study including 1,259 people with asthma reported a moderate VE of 46% in 2012/13 [8, 29].

We found overall protection against influenza in 2013/14 where the influenza activity was low and prolonged, influenza A(H1N1) dominated and the vaccine was well matched [30]. In 2014/15, there was vaccine stain mismatch for H3N2, we observed a positive VE for influenza B similar to findings in a UK-wide study [31]. In the 2015/16 season, our finding of an overall positive VE is consistent with the VE of 55% found in a UK study [32]. The influenza A(H1N1) strain predominated and the vaccine was well matched for this sub-type[33]. We also observed a high VE against influenza B despite lineage mismatch with the vaccine – also found in another study[32].

The overall VE of 46% in children in this study was similar to a recent TND study in Canada where the VE was 43% in children [11]. However, in our study, protection was found only against the B strain while previous studies have shown also protection against A(H1N1) [34-35]. Lower strain-specific protection was observed in older adults (aged $\geq$55 years old) and no protection was found against influenza A strains. Nevertheless, the VE decrease in $\geq$55 years old may only be indicative of immunosenescence and further better powered studies are needed to investigate this phenomenon. There is evidence that the VE in those over the age of 75 may be lower than in those age 65-74 years [36]. The mechanism for this is uncertain, but may be explained by reduced immune responsiveness to historically used influenza antigens in the most elderly [36]. Such evidence has led to the development and introduction of either adjuvanted influenza vaccines or high dose influenza vaccines in this age group. In addition,

the effects of other factors such as the presence of other underlying conditions in older persons could explain the decrease in VE estimates in this age group.

The strengths of this study include the influenza diagnosis based on a test with high predictive value and reduction of any recall or misclassification bias due to documentation of vaccination and medical condition status in high quality electronic medical records [37]. Additionally, the TND study minimised the effects of selection bias due to differential healthcare seeking behaviour between cases and controls by assessing only the prevention of the vaccine against medically-attended influenza. The inclusion of six seasons increased the power of the study allowing the provision of VE estimates for different seasons, strains and patient characteristics. Thus, this study's findings can be generalised to the wider asthma population in Scotland.

Several limitations also need to be considered in this study. The VE in this study assessed only the prevention of influenza. However, vaccinated individuals may have also been benefited by having less severe influenza illness and a subsequent lower risk of a severe asthma attack. Thus, vaccine protection provided by decrease in influenza severity cannot be quantified in this study [14, 16, 38]. Although the general practice electronic health record includes vaccinations taking place in non-general practice settings, there may be some misclassification of vaccination status. Results from the post-hoc analyses need careful interpretation since they were not pre-specified in the protocol of this study. Unmeasured confounders could still have influenced the VE estimates. Future research should assess the confounding effect on VE from TND studies. TND studies offer an elegant way to deal with selection bias related to healthcare-seeking behaviour between cases and control. However, bias may occur due to differences in healthcare seeking behaviour between vaccinated and unvaccinated patients and swab testing may also differ between vaccinated and non-vaccinated patients particularly in non-sentinel settings [39].

This study showed that vaccination can prevent influenza in individuals with asthma presented with ILI in Scottish primary and secondary care settings. While substantial variation in VE was observed among circulating strains and age groups, protection was still observed in most subgroups. There was significant pooled VE when the A(H1N1) dominated that could be explained by the absence of vaccine mismatch over the three seasons [23, 30, 33]. While the lower pooled VE when the A(H3) dominated could be due to vaccine mismatch in most seasons and the intra-seasonal VE waning [27, 30]. Generally, the protection against the A(H3N2) is usually lower compared to A(H1N1) and B which is around 60% or even higher [40]. Thus, evidence from this study reinforces the recommendation for annual seasonal vaccination in asthma. Although, there are current developments towards universal vaccines with better potency, durability and wide protection, these vaccines may not be available for another decade [41]. Thus, monitoring of the effectiveness of current vaccines should be continued. Further adequately powered studies will be needed to monitor the effectiveness of these vaccines in groups of the population at risk of severe influenza and complications such as persons with asthma.

The findings of this study can guide research and policymakers for the provision of a more targeted and effective vaccination programme improving the current protection of the asthma population. Specifically, policy makers and clinicians should consider adjuvant vaccine or high dose influenza vaccine in people with asthma aged 55 years and above [9]. Healthcare providers and people with asthma will also have now a clearer answer regarding the value of the influenza vaccination which is prevention of influenza infection in children and adults with current asthma.

In summary, we provide compelling national evidence over a number of years that influenza vaccination substantially reduces the risk of influenza in people with asthma. There is a need for strategies to boost influenza vaccination uptake in people with asthma.

Author contributions

EV conducted and wrote this study. C. R. S., N. I. L., K. K., C. R., J. M., B. V. W., L. D. R., R. G., T. E., J. S., C. C. B., and A. S. contributed to the conception of the study. K. K., C. R., and T. E. all helped prepare the data and advised on the statistical analysis. All authors contributed to the design of this study. All authors critically revised earlier drafts of this manuscript. All authors approved final version of this manuscript to be published.

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Disclaimer

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Conflict of interest

We declare no competing interests.

References

1. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet, **2011**; 378:1917-30.
2. Centers for Disease Control and Prevention. Flu season. http://www.cdc.gov/flu/about/season/flu-season.htm. Accessed 10 Mar 2015.
3. Schwarze J, Openshaw P, Jha A, et al. Influenza burden, prevention, and treatment in asthma-A scoping review by the EACCI Influenza in asthma task force. Allergy, **2018**; 1-31.
4. Background Paper on Influenza Vaccination and Immunization, SAGE Working Group. http://www.who.int/immunization/sage/meetings/2012/april/1_Background_Paper_Mar26_v13_cleaned.pdf. Accessed 14 Sep 2015.
5. Public Health England. Influenza: the green book. Chapter 19. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/663694/Greenbook_chapter_19_Influenza_.pdf. Accessed 28 Mar 2018.
6. Centers for Disease Control and Prevention. Influenza (Flu). Get Vaccinated. https://www.cdc.gov/flu/consumer/vaccinations.htm. Accessed 01 Apr 2018.
7. Commission of the European Communities. Proposal for a council recommendation on seasonal influenza vaccination 2009. http://ec.europa.eu/health/ph_threats/com/Influenza/docs/seasonflu_rec2009_en.pdf. Accessed 11 May 2018.
8. Vasileiou E, Sheikh A, Butler C, et al. Effectiveness of influenza vaccines in asthma: a systematic review and meta-analysis. Clin Infect Dis, **2017**; 65:1388-95.

9. NHS England. Vaccine ordering for 2018-19 influenza season. https://www.england.nhs.uk/wp-content/uploads/2018/02/vaccine-ordering-18-19-influenza-season-gp-pharm.pdf. Accessed 13 Jul 2018.
10. Suarez-Varela MM, Llopis A, Fernandez-Fabrellas E, et al. Asthma and influenza vaccination in elderly hospitalized patients: Matched case-control study in Spain. J Asthma, **2017**: 1-11.
11. Buchan SA, Chung H, Campitelli MA, et al. Vaccine effectiveness against laboratory-confirmed influenza hospitalizations among young children during the 2010-11 to 2013-14 influenza seasons in Ontario, Canada. PloS one, **2017**; 12:e0187834.
12. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis, **2012**; 12:36-44.
13. De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials. Euro Surveill, **2013**; 18:pii: 20585.
14. Jackson ML, Nelson JC. The test-negative design for estimating influenza vaccine effectiveness. Vaccine, **2013**; 31:2165-8.
15. Foppa IM, Haber M, Ferdinands JM, Shay DK. The case test-negative design for studies of the effectiveness of influenza vaccine. Vaccine, **2013**; 31:3104-9.
16. Sullivan SG, Feng S, Cowling BJ. Potential of the test-negative design for measuring influenza vaccine effectiveness: a systematic review. Expert Rev Vaccines, **2014**; 13:1571-91.

17. Boivin G, Côté S, Déry P, De Serres G, Bergeron MG. Multiplex Real-Time PCR Assay for Detection of Influenza and Human Respiratory Syncytial Viruses. J Clin Microbiol, **2004**; 42:45-51.

18. Simpson CR, Lone NI, Kavanagh K, et al. Evaluating the effectiveness, impact and safety of live attenuated and seasonal inactivated influenza vaccination: protocol for the Seasonal Influenza Vaccination Effectiveness II (SIVE II) study. BMJ Open, **2017**; **7**:e014200.

19. RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL. http://www.rstudio.com/. Accessed 03 Jan 2018.

20. STROBE Statement. Strengthening the reporting of observational studies in epidemiology. https://www.strobe-statement.org/index.php?id=available-checklists. Accessed 13 Jul 2018.

21. RECORD Checklist. Reporting of studies Conducted using Observational Routinely-collected Data. http://www.record-statement.org/checklist.php. Accessed 13 Jul 2018.

22. Pebody R, Hardelid P, Fleming DM, et al. Effectiveness of seasonal 2010/11 and pandemic influenza A(H1N1)2009 vaccines in preventing influenza infection in the United Kingdom: mid-season analysis 2010/11. Euro Surveill, **2011**; 16:pii=19791.

23. Health Protection Agency (HPA). Surveillance of influenza and other respiratory viruses in the UK 2010/11. London: HPA; 2011. http://webarchive.nationalarchives.gov.uk/20140629102627/http:/hpa.org.uk/webc/HPAwebFile/HPAweb_C/1296687414154. Accessed 14 Jun 2017.

24. Health Protection Agency (HPA). Surveillance of influenza and other respiratory pathogens in the UK 2011/12. London: HPA; 2012. http://webarchive.nationalarchives.gov.uk/20140714084352/http:/www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317134705939. Accessed 14 Jun 2017.

25. Pebody R, Andrews N, McMenamin J, et al. Vaccine effectiveness of 2011/12 trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: evidence of waning intra-seasonal protection. Euro Surveill, **2013;** 18:pii: 20389.
26. Ohmit SE, Thompson MG, Petrie JG, et al. Influenza Vaccine Effectiveness in the 2011-2012 Season: Protection Against Each Circulating Virus and the Effect of Prior Vaccination on Estimates. Clin Infect Dis, **2014**; 58:319-27.
27. Andrews N, McMenamin J, Durnall H, et al. Effectiveness of trivalent seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2012/13 end of season results. Euro Surveill, **2014**; 19:pii=20851.
28. Public Health England. Surveillance of influenza and other respiratory viruses, including novel respiratory viruses, in the United Kingdom: Winter 2012/13. London, 2013. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/325217/Annual_flu_report_winter_2012_to_2013.pdf. Accessed 14 Jun 2017.
29. McLean HQ, Thompson MG, Sundaram ME, et al. Influenza vaccine effectiveness in the United States during 2012-2013: variable protection by age and virus type. J Infect Dis, **2015**; 211:1529-40.
30. Public Health England. Surveillance of influenza and other respiratory viruses in the United Kingdom: Winter 2013/14. London, 2014. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/325203/Flu_annual_report_June_2014.pdf. Accessed 14 Jun 2017.
31. Pebody R, Warburton F, Andrews N, et al. Effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 end of season results. Euro Surveill, **2015**; 20:pii=30013.

32. Pebody R, Warburton F, Ellis J, et al. Effectiveness of seasonal influenza vaccine for adults and children in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2015/16 end-of-season results. Euro Surveill, **2016**; 21:pii=30348.
33. Public Health England. Surveillance of influenza and other respiratory viruses in the United Kingdom: Winter 2015/16. London, 2016. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/526405/Flu_Annual_Report_2015_2016.pdf. Accessed 14 Jun 2017.
34. Miyazaki C, Nakayama M, Tanaka Y, et al. Immunization of institutionalized asthmatic children and patients with psychomotor retardation using live attenuated cold-adapted reassortment influenza A H1N1, H3N2 and B vaccines. Vaccine, **1993**; 11:853-8.
35. Tanaka Y, Ueda K, Miyazaki C, et al. Trivalent cold recombinant influenza live vaccine in institutionalized children with bronchial asthma and patients with psychomotor retardation. Pediatr Infect Dis J, **1993**; 12:600-5.
36. Pebody RG, Warburton F, Andrews N, et al. Uptake and effectiveness of influenza vaccine in those aged 65 years and older in the United Kingdom, influenza seasons 2010/11 to 2016/17. Euro Surveill., **2018**; 23:pii=1800092.
37. Jhund PS, MacIntyre K, Simpson CR, et al. Long-term trends in first hospitalization for heart failure and subsequent survival between 1986 and 2003: a population survey of 5.1 million people. Circulation, **2009**; 119:515-23.
38. Simpson CR, Lone NI, Kavanagh K, et al. Trivalent inactivated seasonal influenza vaccine effectiveness for the prevention of laboratory-confirmed influenza in a Scottish population 2000 to 2009. Euro Surveill, **2015**; 20:pii:21043.

39. Lewnard JA, Tedijanto C, Cowling BJ, Lipsitch M. Measurement of Vaccine Direct Effects Under the Test-Negative Design. Am J Epidemiol, **2018**; 187:2686–2697.
40. Monto AS. Moving Toward Improved Influenza Vaccines. J Infect Dis, **2017**; 215:500-2.
41. Berlanda Scorza F, Tsvetnitsky V, Donnelly JJ. Universal influenza vaccines: Shifting to better vaccines. Vaccine, **2016**; 34:2926-33.

FIGURE LEGENDS

Figure 1: Phases of data extraction, linkage and analysis in a secured environment.

Figure 2: Flow diagram for the test-negative design case-control study for an asthma population for the influenza seasons 2010/11 to 2015/16, Scotland, UK.

Figure 3: Vaccine effectiveness against laboratory confirmed overall influenza (Influenza A and B) by season.
*****Season with poorly matched vaccine
^Season with high influenza attack rate

Figure 4: Vaccine effectiveness against laboratory confirmed influenza A(H3) subtype by season.
*****Season with poorly matched vaccine
^Season with high influenza attack rate

Figure 5: Vaccine effectiveness against laboratory confirmed influenza A(H1N1) subtype by season.
*****Season with poorly matched vaccine
^Season with high influenza attack rate

Figure 6: Vaccine effectiveness against laboratory confirmed influenza B subtype by season
*****Season with poorly matched vaccine.
^Season with high influenza attack rate

Table 1*:* Number of influenza (sub)types out of the 781 influenza positive cases

Influenza (sub)types	No. of influenza (sub)types / No. of cases (%)
Influenza A	581/781 (74.4)
A(H1N1)	240/781 (30.7)
A(H3)	208/781 (26.6)
A(unknown)	133/781 (17.0)
Influenza B	205/781 (26.2)
Influenza A & B	5/781 (0.6)

Table 2: Baseline characteristics for cases and controls with asthma during six seasons, Scotland, 2010-16 (n=5,910)

Covariates	Total samples (% of total)	No. Vaccinated at test (% of total)	P value*	No. of positive swabs (% of total)	P value	Swab-positive adjusted OR[a]	Adjusted 95% CI
Gender							
Female (reference)	3,575 (60.5)	1,777 (49.7)	0.04	469 (13.1)	0.79	NA	NA
Male	2,335 (39.5)	1,097 (47.0)		312 (13.4)		1.02	0.88 to 1.19
Age group (years) [b]							
0-1	5 (0.1)	3 (60.0)	<0.001	0 (0.0)	0.0004	8.688845e-06	1.320726e-209 to 5.716258e+198
2-4	169 (2.9)	64 (37.9)		11 (6.5)		0.47	0.25 to 0.90
5-11	530 (9.0)	213 (40.2)		45 (8.5)		0.63	0.44 to 0.91
12-17	371 (6.3)	119 (32.1)		45 (12.1)		0.94	0.65 to 1.35
18-44	1615 (27.3)	436 (27.0)		234 (14.5)		1.15	0.90 to 1.47
45-64	1625 (27.5)	826 (50.8)		246 (15.1)		1.21	0.95 to 1.54
65-74	747 (12.6)	549 (73.5)		91 (12.2)		0.94	0.70 to 1.27
≥75 (reference)	847 (14.3)	663 (78.3)		109 (12.9)		NA	NA
Deprivation quintile[c]							
1[d] (reference)	1350 (22.8)	620 (45.9)	0.06	178 (13.2)	0.69	NA	NA
2	1486 (25.1)	732 (49.3)		184 (12.4)		0.93	0.75 to 1.16
3	1035 (17.5)	531 (51.3)		147 (14.2)		1.09	0.86 to 1.38
4	976 (16.5)	465 (47.6)		130 (13.3)		1.01	0.79 to 1.29
5	938 (15.9)	475 (50.6)		116 (12.4)		0.93	0.72 to 1.19
Urban/rural score[e]							
1 (reference)	3210 (54.3)	1573 (49.0)	0.003	352 (11.0)	<0.001	NA	NA
2	1459 (24.7)	676 (46.3)		228 (15.6)		1.50	1.26 to 1.80
3	381 (6.4)	183 (48.0)		63 (16.5)		1.61	1.19 to 2.14
4	91 (1.5)	40 (44.0)		16 (17.6)		1.73	0.97 to 2.93
5	54 (0.9)	22 (40.7)		14 (25.9)		2.84	1.48 to 5.15
6	448 (7.6)	253 (56.5)		57 (12.7)		1.18	0.87 to 1.58
7	63 (1.1)	24 (38.1)		16 (25.4)		2.76	1.51 to 4.82
8[f]	118 (2.0)	65 (55.1)		17 (14.4)		1.37	0.78 to 2.25
COPD	775 (13.1)	522 (67.4)	<0.001	95 (12.3)	0.40	0.91	0.72 to 1.13

Chronic heart disease	722 (12.2)	527 (73.0)	<0.001	92 (12.7)	0.69	0.95	0.75 to 1.20
Chronic liver disease	112 (1.9)	56 (50.0)	0.77	15 (13.4)	0.96	1.02	0.56 to 1.70
Chronic neurological disease	357 (6.0)	251 (70.3)	<0.001	45 (12.6)	0.73	0.94	0.68 to 1.29
Diabetes	597 (10.1)	417 (69.8)	<0.001	75 (12.6)	0.62	0.94	0.72 to 1.20
Immunosupression	166 (2.8)	85 (51.2)	0.5	18 (10.8)	0.36	0.79	0.47 to 1.27
Number of risk groups (comorbidities)							
1 (reference)	3693 (62.5)	1440 (39.0)	<0.001	490 (13.3)	0.71	NA	NA
2	1042 (17.6)	632 (60.7)		141 (13.5		1.02	0.83 to 1.25
3	705 (11.9)	461 (65.4)		95 (13.5)		1.02	0.80 to 1.28
4	333 (5.6)	241 (72.4)		39 (11.7)		0.87	0.60 to 1.21
5	112 (1.2)	81 (72.3)		11 (9.8)		0.71	0.36 to 1.28
6	25 (0.4)	19 (76.0)		5 (20.0)		1.63	0.54 to 4.06
Influenza vaccine in previous season							
Yes	3352 (56.7)	2417 (72.1)	<0.001	392 (11.7)	<0.001	0.74	0.64 to 0.86
No (reference)	2558 (43.3)	457 (17.9)		389 (15.2)		NA	NA
Swab samples taken in general practices or hospitals							
General practice (reference)	873 (14.8)	359 (41.1)	<0.001	146 (16.7)	0.0005	NA	NA
Hospital	5010 (84.8)	2494 (49.8)		628 (12.5)		0.71	0.59 to 0.87
Unknown	27 (0.5)	21 (77.8)		7 (25.9)		1.74	0.67 to 4.02

Abbreviations: OR: odds ratio; CI: confidence interval; NA: not applicable

a Adjusted for gender, age and socioeconomic deprivation

b Age group available for 5,909 swabs

c Deprivation score only available for 5,785 swabs

d Most socioeconomically deprived

e Urban/rural score only available for 5,824 swabs

f Remote rural areas

* All p-values were estimated using the x^2 test

Table 3: Vaccine effectiveness for laboratory-confirmed influenza type and subtype by season, Scotland, 2010-16

Dominant circulating strain(s) for each influenza season	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total (n)	Vaccinated (%)			
Season: 2010-2011	Influenza A & B	29/123	23.6	176/364	48.4	25.3	70.1 (49.5 to 82.3)	76.1 (55.6 to 87.1)
A/California/07/2009 (H1N1)pdm2009	A(H3)	0/0	0.0	205/487	42.1	0.0	0.0 (-Inf to 100)	0.0 (-Inf to100)
	A(H1N1)	17/79	21.5	188/408	46.1	16.2	68.8 (37.9 to 84.3)	70.7 (32.5 to 87.3)
B/Brisbane/60/2008	Influenza B	5/26	19.2	200/461	43.4	5.3	78.0 (37.3 to 92.3)	83.2 (44.3 to 94.9)
Season: 2011-2012	Influenza A & B	14/28	50.0	241/546	44.1	4.9	34.4 (-44.3 to 70.1)	45.1 (-35.1 to 77.7)
A/Victoria/208/2009 (H3N2)	A(H3)	6/11	54.6	249/563	44.2	1.9	20.1 (-173.0 to 76.6)	3.7 (-240.5 to 75.0)
	A(H1N1)	0/0	0.0	255/574	44.4	0.0	0.0 (-Inf to 100)	0.0 (-Inf to 100)
	Influenza B	2/5	40.0	253/569	44.5	0.9	57.1 (-186.7 to 93.6)	71.8 (-358.1 to 98.3)
Season: 2012-2013	Influenza A & B	50/143	35.0	323/691	46.7	17.2	48.2 (22.2 to 65.5)	45.2 (13.8 to 65.1)
A/Victoria/208/2009 (H3N2)	A(H3)	17/45	37.8	356/789	45.1	5.4	27.9 (-36.3 to 61.9)	38.0 (-25.7 to 69.4)
A/St Petersburg/27/2011 (H1N1)	A(H1N1)	3/17	17.7	370/817	45.3	2.0	79.8 (28.3 to 94.3)	77.5 (9.8 to 94.4)
B/Brisbane/60/2008 B/Brisbane/3/2007 B/Massachusetts/02/2012	Influenza B	18/53	34.0	355/781	45.5	6.4	40.0 (-9.8 to 67.3)	11.7 (-70.7 to 54.3)

Season: 2013-2014	Influenza A & B	26/54	48.2	457/878	52.1	5.8	37.7 (-10.7 to 64.9)	52.3 (6.5 to 75.6)
A/California/07/2009 (H1N1)pdm09	A(H3)	2/6	33.3	481/926	51.9	0.6	65.9 (-105.0 to 94.3)	-3.9 (-1304.5 to 92.3)
	A(H1N1)	18/34	52.9	465/898	51.8	3.7	21.4 (-59.2 to 61.2)	32.0 (-52.2 to 69.6)
	Influenza B	2/5	40.0	481/927	51.9	0.5	45.2 (-259.1 to 91.7)	100 (0 to 100)
Season: 2014-2015	Influenza A & B	122/232	52.6	605/1181	51.2	16.4	36.3 (13.3 to 53.2)	48.6 (27.8 to 63.4)
A/Texas/50/2012 (H3N2) B/Yamagata/16/88	A(H3)	79/140	56.4	648/1273	50.9	9.9	21.1 (-16.0 to 46.4)	26.4 (-12.0 to 51.6)
	A(H1N1)	5/6	83.3	722/1407	51.3	0.4	-290.9 (-3301.3 to 55.1)	-157.0 (-2565.5 to 75.2)
	Influenza B	20/49	40.8	707/1364	51.8	3.5	62.0 (30.3 to 79.3)	77.0 (53.9 to 88.5)
Season: 2015-16	Influenza A & B	85/201	42.3	746/1469	50.8	12.0	54.8 (37.8 to 67.1)	57.8 (40.1 to 70.3)
A/California/07/2009 (H1N1)pdm09	A(H3)	2/6	33.3	829/1664	49.8	0.4	39.0 (-294.0 to 90.5)	78.1 (-102.6 to 97.6)
B/Brisbane/60/2008	A(H1N1)	51/104	49.0	780/1566	49.8	6.2	32.8 (-2.0 to 55.7)	36.7 (-0.6 to 60.2)
	Influenza B	26/67	38.8	805/1603	50.2	4.0	60.9 (33.9 to 76.8)	54.7 (19.5 to 74.5)

Abbreviations: CI: confidence interval; NA: not applicable

a Adjusted for time (i.e. days) only

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. general practice or hospital)

-There are cases with unknown influenza A subtype which explains why the total influenza A(H3) and A(H1N1) samples do not add exactly to the total influenza A samples

Table 4: Vaccine effectiveness for laboratory-confirmed influenza by various age groups, Scotland, 2010-16 (n=5,910)

Age (years)	Influenza types & subtypes	Influenza-positive (cases)		Influenza-negative (controls)		Total positive (%)	Unadjusted vaccine effectiveness[a] (95% CI)	Adjusted vaccine Effectiveness[b] (95% CI)
		Vaccinated/ total (n)	Vaccinated (%)	Vaccinated/ total(n)	Vaccinated (%)			
All ages	Influenza A & B	326/781	41.7	2548/5129	49.7	13.2	48.6 (39.2 to 56.6)	55.0 (45.8 to 62.7)
	A(H3)	106/208	51.0	2768/5701	48.5	3.5	26.0 (-0.8 to 45.6)	33.8 (6.7 to 53.1)
	A(H1N1)	94/240	39.2	2780/5670	49.0	4.1	43.2 (23.6 to 57.8)	46.6 (25.4 to 61.8)
	Influenza B	73/205	35.6	2801/5705	49.1	3.5	59.0 (44.2 to 70.0)	61.5 (45.7 to 72.7)
≤17	Influenza A & B	31/101	30.7	368/974	37.8	9.4	52.9 (23.4 to 71.0)	46.0 (11.2 to 67.2)
	A(H3)	8/26	30.8	391/1049	37.3	2.4	55.7 (-11.0 to 82.3)	51.1 (-25.4 to 80.9)
	A(H1N1)	4/15	26.7	395/1060	37.3	1.4	64.9 (-66.8 to 92.6)	90.5 (-45.3 to 99.4)
	Influenza B	12/45	26.7	387/1030	37.6	4.2	69.6 (26.1 to 87.5)	56.3 (3.8 to 80.2)
18-54	Influenza A & B	94/376	25.0	733/2093	35.0	15.2	54.0 (39.2 to 65.2)	57.0 (42.3 to 68.0)
	A(H3)	22/84	26.2	805/2385	33.8	3.4	58.4 (28.4 to 75.8)	53.3 (17.9 to 73.5)
	A(H1N1)	33/143	23.1	794/2326	34.1	5.8	45.7 (14.4 to 65.5)	53.0 (23.8 to 71.1)
	Influenza B	22/89	24.7	805/2380	33.8	3.6	49.9 (15.9 to 70.1)	54.5 (21.1 to 73.7)
55-64	Influenza A & B	51/104	49.0	384/667	57.6	13.5	51.1 (22.0 to 69.4)	57.6 (29.6 to 74.5)

	A(H3)	18/29	62.1	417/742	56.2	3.8	2.6 (-145.9 to 61.4)	2.1 (-178.5 to 65.6)
	A(H1N1)	17/33	51.5	418/738	56.6	4.3	38.0 (-36.3 to 71.8)	38.7 (-43.4 to 73.8)
	Influenza B	7/24	29.2	428/747	57.3	3.1	78.7 (45.0 to 91.8)	88.2 (61.2 to 96.4)
65-74	Influenza A & B	61/91	67.0	488/656	74.4	12.2	54.8 (22.5 to 73.6)	56.8 (24.0 to 74.9)
	A(H3)	18/24	75.0	531/723	73.4	3.2	-13.4 (-249.3 to 63.2)	1.0 (-196.9 to 67.0)
	A(H1N1)	22/30	73.3	527/717	73.5	4.0	57.5 (-37.4 to 86.9)	60.5 (-37.9 to 88.7)
	Influenza B	12/20	60.0	537/727	73.9	2.7	65.3 (9.0 to 86.8)	65.8 (5.2 to 87.6)
≥75	Influenza A & B	89/109	81.7	575/739	77.8	12.9	48.9 (4.8 to 72.5)	51.9 (9.2 to 74.5)
	A(H3)	40/45	88.9	624/803	77.7	5.3	-13.5 (-232.9 to 61.3)	-15.4 (-278.2 to 64.8)
	A(H1N1)	18/19	94.7	646/829	77.9	2.2	-542.3 (-6752.7 to 39.8)	-501.0 (-5639.5 to 37.1)
	Influenza B	20/27	74.1	644/821	78.4	3.2	67.6 (15.1 to 87.6)	70.4 (19.8 to 89.1)

Abbreviations: CI: confidence interval

a Adjusted for time within a season (i.e. days)

b Adjusted for time (i.e. days), age, number of risk groups, swab location (i.e. GP or hospital)

Figure 1

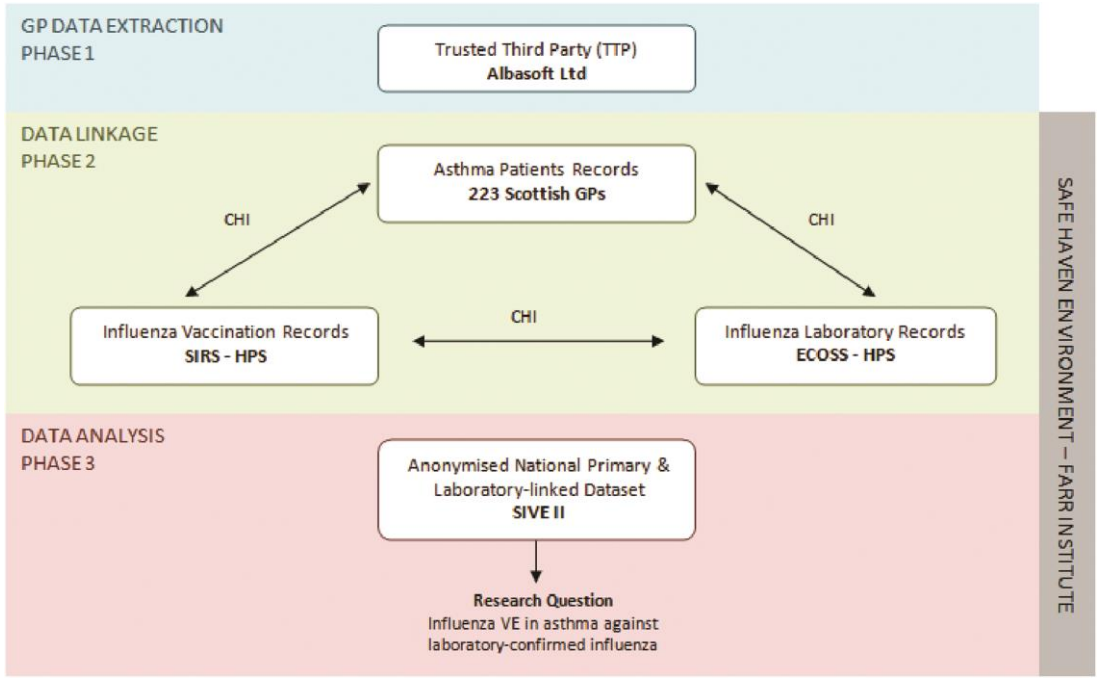

Figure 2

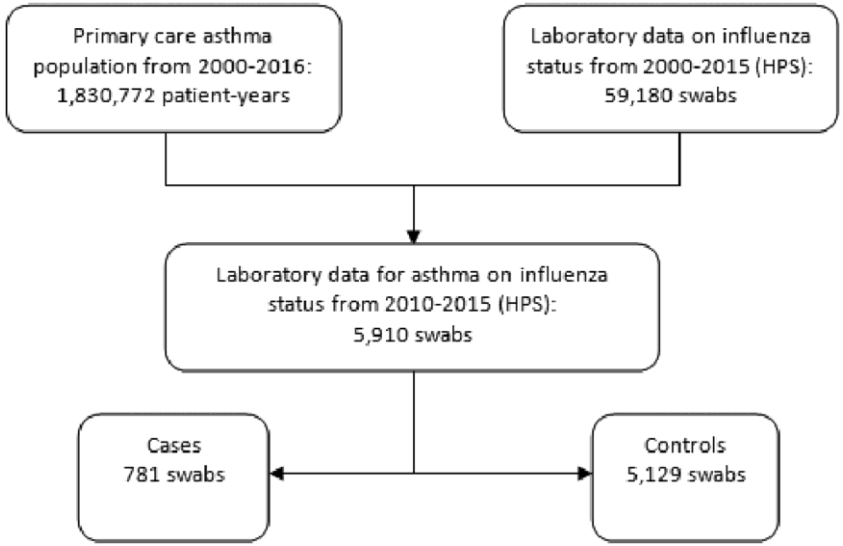

Figure 3

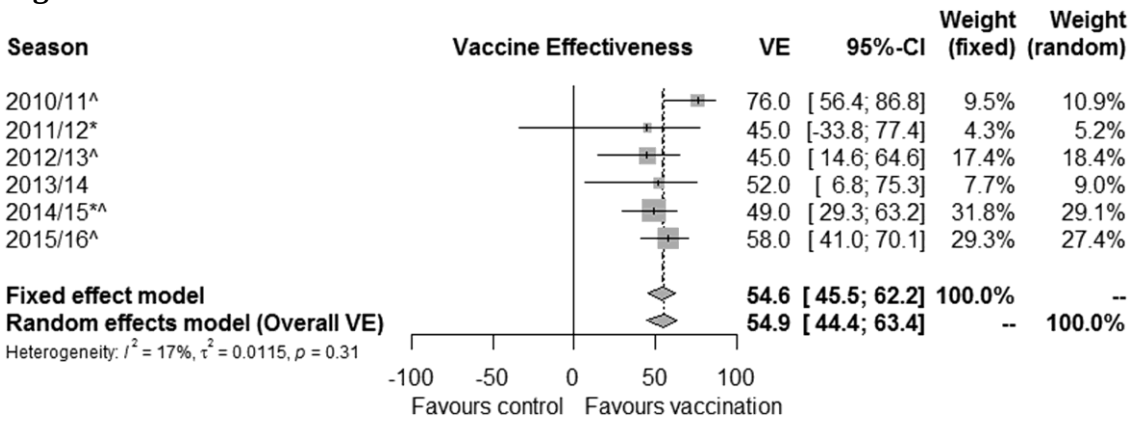

Figure 4

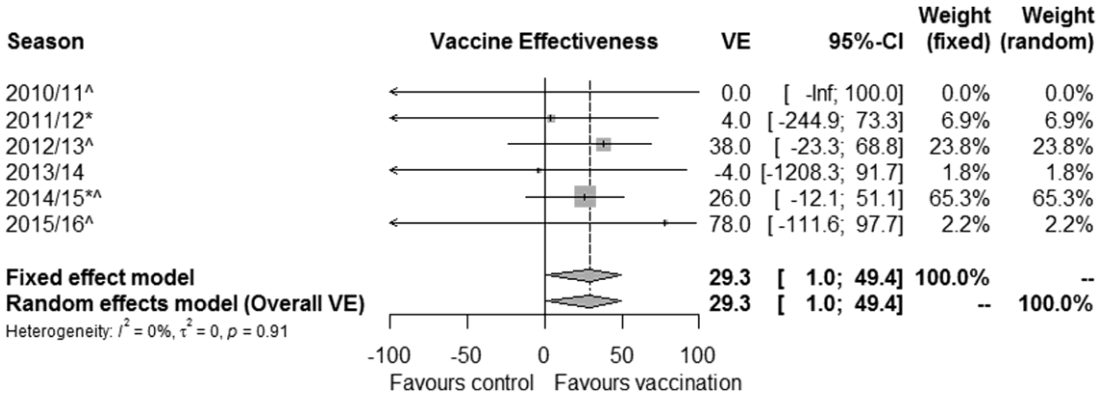

Figure 5

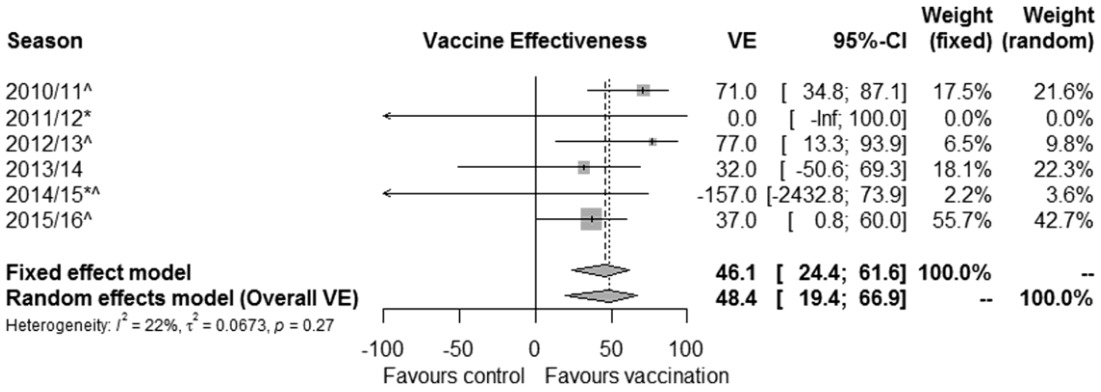

Figure 6